Litton BIONETICS

MUTAGENICITY EVALUATION OF FDA 75-67 ZINC GLUCONATE

FINAL REPORT

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Mutagenic Evaluation of FDA 75-67 **Zinc Gluconate**-Final Report-4/77

MUTAGENICITY EVALUATION
OF
FDA 75-67
ZINC GLUCONATE

FINAL REPORT

SUBMITTED TO

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-67, Zinc Gluconate, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: November 24, 1976

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound FDA 75-67 Zinc Gluconate

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: October 29, 1976

2. Description: White powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-67
Zinc Gluconate

2. Test solvent: Saline

3. Solubility of the test compound under treatment conditions:
Soluble

4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: November 4, 1976

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.02	1.25
1/2 50% Survival	0.04	2.50
50% Survival	0.08	5.00

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 004468024

B. TEST DATE: NOV. 13, 1976

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	22	32	30	25	23	37	39	41	188	195
POSITIVE CONTROL**		---	---	>1000	>1000	>1000	630	>1000	>1000	899	644	986	>1000
TEST	800.00000 %	---	---	10	17	26	24	17	10	26	26	114	121
	400.00000 %	---	---	15	11	27	19	28	19	35	23	134	147
	200.00000 %	---	---	11	25	24	28	24	33	36	27	159	170
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	22	27	30	25	38	29	61	52	207	201
		RAT	LIVER	33	36	33	26	30	37	41	45	279	330
		MONKEY	LIVER	30	27	24	30	34	22	74	56	263	224
POSITIVE CONTROL***		MOUSE	LIVER	878	888	406	588	>1000	>1000	>1000	>1000	500	652
		RAT	LIVER	472	554	819	510	>1000	>1000	>1000	>1000	421	736
		MONKEY	LIVER	693	851	300	653	>1000	>1000	900	>1000	674	654
TEST	0.08%	MOUSE	LIVER	12	12	32	35	36	25	40	59	130	159
	0.04%	MOUSE	LIVER	11	12	25	31	16	25	49	45	126	176
	0.02%	MOUSE	LIVER	10	20	38	36	29	20	47	45	183	176
	0.08%	RAT	LIVER	18	12	30	30	25	34	45	47	100	187
	0.04%	RAT	LIVER	14	13	35	35	27	36	42	46	129	122
	0.02%	RAT	LIVER	17	19	24	37	36	26	31	39	127	185
	0.08%	MONKEY	LIVER	11	17	25	38	26	36	40	58	141	142
	0.04%	MONKEY	LIVER	11	11	22	39	24	31	56	31	189	195
	0.02%	MONKEY	LIVER	14	23	29	35	20	35	42	52	197	194

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES / NONACTIVATION COMPOUND 004468024

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		68.05	9.20	8.44	8.90	7.95	16.12	4.71	
NAP		532.51	182.08	82.18	87.98	143.98	106.27	69.28	CONTROLS
NA1		82.67	13.55	9.80	9.36	5.96	12.84	5.26	
NA2		66.23	14.49	8.87	8.79	7.33	16.56	3.31	TEST COMPOUND
NA3		76.24	14.98	6.48	6.58	6.50	13.56	3.85	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES ICRFLO/MOUSE COMPOUND 004468024

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	136.41	15.43	3.74	5.62	21.75		9.30	3.10	
ACT	A-C	220.44	17.90	13.87	4.11	23.98		14.24	4.75	
ACT	ALI	118.98	7.04	22.76	5.23	41.75	32.19	6.65	3.82	NEGATIVE CONTROLS
ACT	ALU	164.24	9.09	30.58	6.41	58.25	16.22	10.43	3.63	
ACT	PLI	143.72	195.15	54.22	149.81	583.71		96.26	85.59	
ACT	PLU	114.22	10.16	5.84	104.47	215.84		13.76	4.23	POSITIVE CONTROLS
ACT	LI1	102.13	3.64	20.29	8.75	77.96	26.99	8.93	2.98	
ACT	LI2	105.88	4.60	18.01	8.91	51.83		8.40	4.80	
ACT	LI3	76.60	3.83	19.18	9.19	49.07		7.83	2.56	
ACT	LU1	106.67	4.21	30.17	12.50	51.42		9.89	2.83	TEST COMPOUND
ACT	LU2	74.61	3.14	25.60	5.94	99.71	12.55	9.22	2.81	
ACT	LU3	65.44	3.70	25.21	4.21	41.42		8.14	2.71	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES SPRDAW/RAT COMPOUND 004468024

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	38.07	10.26	13.96	12.33	4.80	17.13	5.71	
ACT	A-C	60.38	15.10	6.20	11.48	6.36	14.16	4.72	NEGATIVE CONTROLS
ACT	ALI	56.09	9.92	39.15	9.61	11.00	8.99	3.00	
ACT	ALU	59.81	8.96	19.84	10.02	9.97	12.32	4.11	
ACT	PLI	102.12	104.55	104.69	90.18	240.28	120.70	57.65	POSITIVE CONTROLS
ACT	PLU	92.31	11.74	74.23	111.95	121.82	8.66	2.89	
ACT	LI1	69.59	10.42	24.09	9.00	7.13	6.51	2.17	
ACT	LI2	50.96	8.73	15.85	11.35	9.54	7.45	2.48	
ACT	LI3	52.96	9.17	15.76	8.13	20.53	10.89	3.63	TEST COMPOUND
ACT	LU1	73.10	8.39	11.76	9.11	8.17	10.09	3.36	
ACT	LU2	61.57	10.43	18.55	8.84	8.70	9.34	2.78	
ACT	LU3	56.81	8.60	10.06	11.54	7.23	12.93	4.31	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 11/24/76

SPECIES RHESUS/MONKEY COMPOUND 004468024

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	77.24	8.06	6.31	5.51	5.62	10.32	5.96	
ACT	A-C	83.51	10.35	3.37	3.32	6.17	18.61	5.45	
ACT	ALI	70.32	8.62	25.25	5.02	18.97	8.74	4.14	NEGATIVE CONTROLS
ACT	ALU	78.51	6.90	27.37	7.49	14.80	13.99	5.73	
ACT	PLI	186.02	79.75	95.20	197.18	806.40	104.94	61.24	POSITIVE CONTROLS
ACT	PLU	96.52	9.15	16.84	7.62	11.87	11.24	4.23	
ACT	LI1	82.93	7.03	28.94	4.23	15.44	11.26	8.30	
ACT	LI2	77.23	7.71	16.84	5.18	14.81	4.11	3.35	
ACT	LI3	86.67	7.68	13.72	4.89	13.12	8.56	4.03	TEST COMPOUND
ACT	LU1	79.12	6.94	6.55	8.04	23.87	10.36	3.39	
ACT	LU2	85.46	6.95	15.43	6.07	24.42	9.09	3.14	
ACT	LU3	97.33	8.68	22.61	7.11	18.27	8.12	3.84	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU or A+T = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-67, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. *Salmonella typhimurium*

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. The compound exhibited slightly increased revertant frequency with TA-98 at LI1 and LU2 doses when mouse liver microsomes were used. The repeat tests were negative.

B. *Saccharomyces cerevisiae*

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests negative.

C. Conclusions

The test compound, FDA 75-67, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

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Director — Date
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D. — 3/31/77
Vice President — Date

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

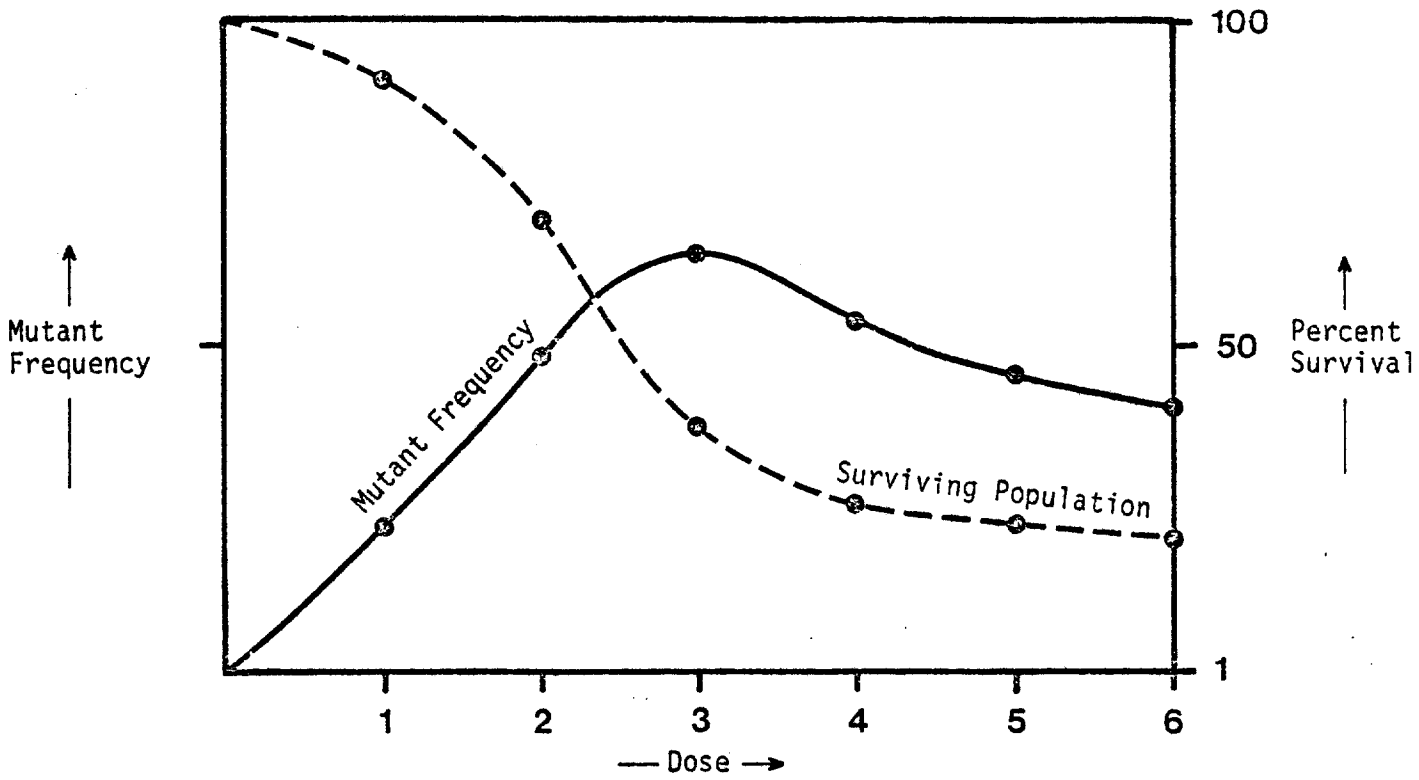
A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 631704 CONTRACT 22374-2104 DETECTOR TA100 SPECIES PROJECT 02468 / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0457	0311	68.05	0
	NAP		EMS 0.066%	0486	2588	532.51	0
004468024	NA1		0008-2 PCT.	0375	0310	82.67	0
004468024	NA2		0004-2 PCT.	0456	0302	66.23	0
004468024	NA3		0002-2 PCT.	0484	0369	76.24	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631707 DETECTOR TA1535 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0652	0060	9.20	0
	NAP		EMS 0.2%	0558	1016	182.08	0
004468024	NA1		0008-2 PCT.	0738	0100	13.55	0
004468024	NA2		0004-2 PCT.	0690	0100	14.49	0
004468024	NA3		0002-2 PCT.	0654	0098	14.98	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631502 DETECTOR TA1537 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1729	0146	8.44	0
	NAP		QM 13 UG/ML	1027	0844	82.18	0
004468024	NA1		0008-2 PCT.	1520	0149	9.80	0
004468024	NA2		0004-2 PCT.	1905	0169	8.87	0
004468024	NA3		0002-2 PCT.	2267	0147	6.48	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631705 DETECTOR TA1538 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0427	0038	8.90	0
	NAP		NF 667 UG/ML	0391	0344	87.98	0
004468024	NA1		0008-2 PCT.	0406	0038	9.36	0
004468024	NA2		0004-2 PCT.	0387	0034	8.79	0
004468024	NA3		0002-2 PCT.	0395	0026	6.58	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631708 DETECTOR TA98 SPECIES / DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1270	0101	7.95	0
	NAP		NF 667 UG/ML	0864	1244	143.98	0
004468024	NA1		0008-2 PCT.	1560	0093	5.96	0
004468024	NA2		0004-2 PCT.	1268	0093	7.33	1
004468024	NA3		0002-2 PCT.	1215	0079	6.50	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632302 DETECTOR 000004 SPECIES / DATE - 11/24/76

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0552	0089	0026	16.12	4.71	0
	NAP		EMS 1.0 %	0638	0678	0442	106.27	69.28	0
004468024	NA1		0005-0 PCT.	0475	0061	0025	12.84	5.26	0
004468024	NA2		0025-1 PCT.	0453	0075	0015	16.56	3.31	0
004468024	NA3		0125-2 PCT.	0494	0067	0019	13.56	3.85	0

REPORT EXH33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632007 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0206	0281	136.41	1
	A-C		SOLVENT	0137	0302	220.44	1
	ALI		TISSUE	0216	0257	118.98	1
	ALU		TISSUE	0165	0271	164.24	0
	ACP	LI	DMN 90 UM/ML	0183	0263	143.72	0
	ACP	LU	DMN 90 UM/ML	0204	0233	114.22	0
004468024	ACT	LI1	0008-2 PCT.	0375	0383	102.13	0
004468024	ACT	LI2	0004-2 PCT.	0238	0252	105.88	0
004468024	ACT	LI3	0002-2 PCT.	0312	0239	76.60	0
004468024	ACT	LU1	0008-2 PCT.	0270	0288	106.67	0
004468024	ACT	LU2	0004-2 PCT.	0256	0191	74.61	0
004468024	ACT	LU3	0002-2 PCT.	0353	0231	65.44	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632102 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0350	0054	15.43	0
	A-C		SOLVENT	0352	0063	17.90	0
	ALI		TISSUE	0398	0028	7.04	0
	ALU		TISSUE	0385	0035	9.09	0
	ACP	LI	DMN 90 UM/ML	0536	1046	195.15	1
	ACP	LU	DMN 90 UM/ML	0364	0037	10.16	0
004468024	ACT	LI1	0008-2 PCT.	0385	0014	3.64	0
004468024	ACT	LI2	0004-2 PCT.	0435	0020	4.60	0
004468024	ACT	LI3	0002-2 PCT.	0392	0015	3.83	0
004468024	ACT	LU1	0008-2 PCT.	0404	0017	4.21	0
004468024	ACT	LU2	0004-2 PCT.	0382	0012	3.14	0
004468024	ACT	LU3	0002-2 PCT.	0378	0014	3.70	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631706 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1204	0045	3.74	0
	A-C		SOLVENT	1298	0180	13.87	0
	ALI		TISSUE	1015	0231	22.76	0
	ALU		TISSUE	0798	0244	30.58	0
	ACP	LI	AMQ 333 UG/ML	1741	0944	54.22	0
	ACP	LU	AMQ 333 UG/ML	0908	0053	5.84	0
004468024	ACT	LI1	0008-2 PCT.	1227	0249	20.29	0
004468024	ACT	LI2	0004-2 PCT.	1166	0210	18.01	0
004468024	ACT	LI3	0002-2 PCT.	1126	0216	19.18	0
004468024	ACT	LU1	0008-2 PCT.	0643	0194	30.17	0
004468024	ACT	LU2	0004-2 PCT.	0668	0171	25.60	0
004468024	ACT	LU3	0002-2 PCT.	0714	0180	25.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632005 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0498	0028	5.62	0
	A-C		SOLVENT	0560	0023	4.11	0
	ALI		TISSUE	0554	0029	5.23	2
	ALU		TISSUE	0515	0033	6.41	2
	ACP	LI	ANTH 67 UG/ML	0532	0797	149.81	0
	ACP	LU	ANTH 67 UG/ML	0559	0584	104.47	0
004468024	ACT	LI1	0008-2 PCT.	0526	0046	8.75	2
004468024	ACT	LI2	0004-2 PCT.	0494	0044	8.91	0
004468024	ACT	LI3	0002-2 PCT.	0479	0044	9.19	0
004468024	ACT	LU1	0008-2 PCT.	0440	0055	12.50	0
004468024	ACT	LU2	0004-2 PCT.	0471	0028	5.94	0
004468024	ACT	LU3	0002-2 PCT.	0475	0020	4.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 631801 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH .67 UG/ML	1108	0241	21.75	0
	A-C		SOLVENT	1051	0252	23.98	0
	ALI		TISSUE	0582	0243	41.75	0
	ALU		TISSUE	0424	0247	58.25	0
	ACP	LI	ANTH 67 UG/ML	0356	2078	583.71	0
	ACP	LU	ANTH 67 UG/ML	0221	0477	215.84	0
004468024	ACT	LI1	0008-2 PCT.	0363	0283	77.96	0
004468024	ACT	LI2	0004-2 PCT.	0218	0113	51.83	0
004468024	ACT	LI3	0002-2 PCT.	0377	0185	49.07	0
004468024	ACT	LU1	0008-2 PCT.	0387	0199	51.42	0
004468024	ACT	LU2	0004-2 PCT.	0349	0348	99.71	0
004468024	ACT	LU3	0002-2 PCT.	0688	0285	41.42	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632407 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	ALI		TISSUE	0963	0310	32.19	0
	ALU		TISSUE	2010	0326	16.22	0
00446A024	ACT	LI1	0008-2 PCT.	1256	0339	26.99	0
00446A024	ACT	LU2	0004-2 PCT.	2463	0309	12.55	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632027 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0774	0072	0024	9.30	3.10	0
	A-C		SOLVENT	0674	0096	0032	14.24	4.75	0
	ALI		TISSUE	0812	0054	0031	6.65	3.82	0
	ALU		TISSUE	0633	0066	0023	10.43	3.63	0
	ACP	LI	DMN 90 UM/ML	0562	0541	0481	96.26	85.59	0
	ACP	LU	DMN 90 UM/ML	0567	0078	0024	13.76	4.23	0
004468024	ACT	LI1	0005-0 PCT.	0571	0051	0017	8.93	2.98	0
004468024	ACT	LI2	0025-1 PCT.	0750	0063	0036	8.40	4.80	0
004468024	ACT	LI3	0125-2 PCT.	0664	0052	0017	7.83	2.56	0
004468024	ACT	LU1	0005-0 PCT.	0566	0056	0016	9.89	2.83	0
004468024	ACT	LU2	0025-1 PCT.	0640	0059	0018	9.22	2.81	0
004468024	ACT	LU3	0125-2 PCT.	0663	0054	0018	8.14	2.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632008 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0746	0284	38.07	0
	A-C		SOLVENT	0530	0320	60.38	0
	ALI		TISSUE	0517	0290	56.09	0
	ALU		TISSUE	0525	0314	59.81	0
	ACP	LI	DMN 90 UM/ML	0661	0675	102.12	0
	ACP	LU	DMN 90 UM/ML	0325	0300	92.31	0
004468024	ACT	LI1	0008-2 PCT.	0319	0222	69.59	0
004468024	ACT	LI2	0004-2 PCT.	0363	0185	50.96	0
004468024	ACT	LI3	0002-2 PCT.	0321	0170	52.96	0
004468024	ACT	LU1	0008-2 PCT.	0368	0269	73.10	0
004468024	ACT	LU2	0004-2 PCT.	0484	0298	61.57	0
004468024	ACT	LU3	0002-2 PCT.	0653	0371	56.81	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632101 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0468	0048	10.26	0
	A-C		SOLVENT	0457	0069	15.10	0
	ALI		TISSUE	0484	0048	9.92	0
	ALU		TISSUE	0491	0044	8.96	0
	ACP	LI	DMN 90 UM/ML	0571	0597	104.55	0
	ACP	LU	DMN 90 UM/ML	0426	0050	11.74	0
004468024	ACT	LI1	0008-2 PCT.	0432	0045	10.42	0
004468024	ACT	LI2	0004-2 PCT.	0561	0049	8.73	0
004468024	ACT	LI3	0002-2 PCT.	0480	0044	9.17	0
004468024	ACT	LU1	0008-2 PCT.	0465	0039	8.39	0
004468024	ACT	LU2	0004-2 PCT.	0422	0044	10.43	0
004468024	ACT	LU3	0002-2 PCT.	0558	0048	8.60	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632002 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMO 333 UG/ML	0695	0097	13.96	0
	A-C		SOLVENT	1371	0085	6.20	0
	ALI		TISSUE	0544	0213	39.15	0
	ALU		TISSUE	0731	0145	19.84	0
	ACP	LI	AMO 333 UG/ML	0640	0670	104.69	0
	ACP	LU	AMO 333 UG/ML	0710	0527	74.23	0
004468024	ACT	LI1	0008-2 PCT.	0303	0073	24.09	0
004468024	ACT	LI2	0004-2 PCT.	0593	0094	15.85	0
004468024	ACT	LI3	0002-2 PCT.	0406	0064	15.76	0
004468024	ACT	LU1	0008-2 PCT.	0612	0072	11.76	0
004468024	ACT	LU2	0004-2 PCT.	0512	0095	18.55	0
004468024	ACT	LU3	0002-2 PCT.	0785	0079	10.06	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632009 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0576	0071	12.33	0
	A-C		SOLVENT	0636	0073	11.48	0
	ALI		TISSUE	0458	0044	9.61	0
	ALU		TISSUE	0449	0045	10.02	0
	ACP	LI	ANTH 67 UG/ML	0448	0404	90.18	0
	ACP	LU	ANTH 67 UG/ML	0452	0506	111.95	0
004468024	ACT	LI1	0008-2 PCT.	0522	0047	9.00	0
004468024	ACT	LI2	0004-2 PCT.	0458	0052	11.35	0
004468024	ACT	LI3	0002-2 PCT.	0566	0046	8.13	0
004468024	ACT	LU1	0008-2 PCT.	0505	0046	9.11	0
004468024	ACT	LU2	0004-2 PCT.	0464	0041	8.84	0
004468024	ACT	LU3	0002-2 PCT.	0468	0054	11.54	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632003 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1790	0086	4.80	0
	A-C		SOLVENT	1808	0115	6.36	0
	ALI		TISSUE	1045	0115	11.00	0
	ALU		TISSUE	1003	0100	9.97	0
	ACP	LI	ANTH 67 UG/ML	0576	1384	240.28	0
	ACP	LU	ANTH 67 UG/ML	1054	1284	121.82	0
004468024	ACT	LI1	0008-2 PCT.	1600	0114	7.13	0
004468024	ACT	LI2	0004-2 PCT.	1174	0112	9.54	0
004468024	ACT	LI3	0002-2 PCT.	0604	0124	20.53	0
004468024	ACT	LU1	0008-2 PCT.	1371	0112	8.17	0
004468024	ACT	LU2	0004-2 PCT.	1356	0118	8.70	0
004468024	ACT	LU3	0002-2 PCT.	1341	0097	7.23	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632304 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0648	0111	0037	17.13	5.71	0
	A-C		SOLVENT	0657	0093	0031	14.16	4.72	0
	ALI		TISSUE	0567	0051	0017	8.99	3.00	0
	ALU		TISSUE	0609	0075	0025	12.32	4.11	0
	ACP	LI	DMN 90 UM/ML	0739	0892	0426	120.70	57.65	0
	ACP	LU	DMN 90 UM/ML	0658	0057	0019	8.66	2.89	0
004468024	ACT	LI1	0005-0 PCT.	0507	0033	0011	6.51	2.17	0
004468024	ACT	LI2	0025-1 PCT.	0483	0036	0012	7.45	2.48	0
004468024	ACT	LI3	0125-2 PCT.	0496	0054	0018	10.89	3.63	0
004468024	ACT	LU1	0005-0 PCT.	0446	0045	0015	10.09	3.36	0
004468024	ACT	LU2	0025-1 PCT.	0503	0047	0014	9.34	2.78	1
004468024	ACT	LU3	0125-2 PCT.	0673	0087	0029	12.93	4.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632104 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0312	0241	77.24	0
	A-C		SOLVENT	0279	0233	83.51	0
	ALI		TISSUE	0310	0218	70.32	0
	ALU		TISSUE	0335	0263	78.51	0
	ACP	LI	DMN 90 UM/ML	0322	0599	186.02	0
	ACP	LU	DMN 90 UM/ML	0230	0222	96.52	0
004468024	ACT	LI1	0008-2 PCT.	0369	0306	82.93	0
004468024	ACT	LI2	0004-2 PCT.	0325	0251	77.23	0
004468024	ACT	LI3	0002-2 PCT.	0300	0260	86.67	0
004468024	ACT	LU1	0008-2 PCT.	0364	0288	79.12	0
004468024	ACT	LU2	0004-2 PCT.	0337	0288	85.46	0
004468024	ACT	LU3	0002-2 PCT.	0337	0328	97.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632103 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0633	0051	8.06	0
	A-C		SOLVENT	0570	0059	10.35	0
	ALI		TISSUE	0580	0050	8.62	0
	ALU		TISSUE	0623	0043	6.90	0
	ACP	LI	DMN 90 UM/ML	0632	0504	79.75	0
	ACP	LU	DMN 90 UM/ML	0568	0052	9.15	0
004468024	ACT	LI1	0008-2 PCT.	0640	0045	7.03	0
004468024	ACT	LI2	0004-2 PCT.	0597	0046	7.71	0
004468024	ACT	LI3	0002-2 PCT.	0638	0049	7.68	0
004468024	ACT	LU1	0008-2 PCT.	0620	0043	6.94	0
004468024	ACT	LU2	0004-2 PCT.	0604	0042	6.95	0
004468024	ACT	LU3	0002-2 PCT.	0553	0048	8.68	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632001 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1141	0072	6.31	0
	A-C		SOLVENT	1750	0059	3.37	0
	ALI		TISSUE	0713	0180	25.25	0
	ALU		TISSUE	0676	0185	27.37	0
	ACP	LI	AMQ 333 UG/ML	0771	0734	95.20	0
	ACP	LU	AMQ 333 UG/ML	0392	0066	16.84	0
004468024	ACT	LI1	0008-2 PCT.	0273	0079	28.94	0
004468024	ACT	LI2	0004-2 PCT.	0392	0066	16.84	0
004468024	ACT	LI3	0002-2 PCT.	0452	0062	13.72	0
004468024	ACT	LU1	0008-2 PCT.	0916	0060	6.55	0
004468024	ACT	LU2	0004-2 PCT.	0512	0079	15.43	0
004468024	ACT	LU3	0002-2 PCT.	0283	0064	22.61	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632010 DETECTION TA1538 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0490	0027	5.51	0
	A-C		SOLVENT	0542	0018	3.32	0
	ALI		TISSUE	0438	0022	5.02	0
	ALU		TISSUE	0414	0031	7.49	0
	ACP	LI	ANTH 67 UG/ML	0674	1329	197.18	0
	ACP	LU	ANTH 67 UG/ML	0564	0043	7.62	0
004468024	ACT	LI1	0008-2 PCT.	0449	0019	4.23	0
004468024	ACT	LI2	0004-2 PCT.	0502	0026	5.18	0
004468024	ACT	LI3	0002-2 PCT.	0470	0023	4.89	0
004468024	ACT	LU1	0008-2 PCT.	0460	0037	8.04	0
004468024	ACT	LU2	0004-2 PCT.	0461	0028	6.07	0
004468024	ACT	LU3	0002-2 PCT.	0464	0033	7.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632004 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	2027	0114	5.62	0
	A-C		SOLVENT	1134	0070	6.17	0
	ALI		TISSUE	0738	0140	18.97	0
	ALU		TISSUE	0939	0139	14.80	2
	ACP	LI	ANTH 67 UG/ML	0422	3403	806.40	0
	ACP	LU	ANTH 67 UG/ML	1070	0127	11.87	3
004468024	ACT	LI1	0008-2 PCT.	0868	0134	15.44	0
004468024	ACT	LI2	0004-2 PCT.	0844	0125	14.81	0
004468024	ACT	LI3	0002-2 PCT.	1067	0140	13.12	0
004468024	ACT	LU2	0004-2 PCT.	0991	0242	24.42	0
004468024	ACT	LU3	0002-2 PCT.	1478	0270	18.27	0
004468024	ACT	LU1	0008-2 PCT.	0926	0221	23.87	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 632301 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 11/24/76

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0688	0071	0041	10.32	5.96	0
	A-C		SOLVENT	0532	0099	0029	18.61	5.45	0
	ALI		TISSUE	0652	0057	0027	8.74	4.14	0
	ALU		TISSUE	0715	0100	0041	13.99	5.73	0
	ACP	LI	DMN 90 UM/ML	0627	0658	0384	104.94	61.24	0
	ACP	LU	DMN 90 UM/ML	0685	0077	0029	11.24	4.23	0
004468024	ACT	LI1	0005-0 PCT.	0506	0057	0042	11.26	8.30	0
004468024	ACT	LI2	0025-1 PCT.	0657	0027	0022	4.11	3.35	0
004468024	ACT	LI3	0125-2 PCT.	0596	0051	0024	8.56	4.03	0
004468024	ACT	LU1	0005-0 PCT.	0560	0058	0019	10.36	3.39	0
004468024	ACT	LU2	0025-1 PCT.	0605	0055	0019	9.09	3.14	0
004468024	ACT	LU3	0125-2 PCT.	0677	0055	0026	8.12	3.84	0